Summary

The work described in this thesis was aimed at understanding the cellular processes involved in protein aggregation and how this relates to human diseases. The work includes the identification and characterisation of MOAG-4 and its human orthologs SERF1a and SERF2, proteins found to drive protein aggregation, and the development of methods to study protein misfolding and aggregation.

In chapter 2 MOAG-4 and SERF1A and SERF2 were found to be small, intrinsically unfolded proteins that promote protein aggregation. Their function is conserved between *Caenorhabditis elegans* and humans as is the amino acid sequence and overall positive charge. Differences in sequence or context have not revealed any significant differences in the function between Moag-4, SERF1A and SERF2. As differences in the subcellular distribution has been found for SERF1 and SERF2. It is reasonable to assume that some differences in function will eventually be found.

Although little is known about of the pathway in which MOAG-4 and the SERF proteins act, the protective effect of its removal in *C. elegans* seems independent of the heat shock response and of protein degradation machineries. Even more, the removal of the heat shock transcription factor 1 (HSF-1), molecular chaperones or factors required for protein degradation do no longer lead to a significant increase in protein aggregation in the absence of MOAG-4, whereas it does in its presence. Conversely, MOAG-4 does not seem to be part of the heat shock response, as we could not observe any changes in expression of MOAG-4 upon heat shock, despite the presence of several putative HSF-1 binding sites within 5000 base pairs upstream of the *moag-4* gene.

Given that SERF and MOAG are sufficient to promote amyloid specific aggregation *in vitro* without the need for other factors, these observations suggest that MOAG-4 and the SERF proteins may act downstream of these protein quality control systems to drive aggregation prone proteins into aggregates. The strong dependence of protein aggregation in *C. elegans* on MOAG-4 indicates a lack of alternative pathways in protein aggregation. One possibility is that MOAG-4 acts as the only significant gateway to active amyloid protein sequestration. It is still unknown to what extent this is true for mammalian systems which express SERF orthologues from several chromosomal loci. The strength of the gateway effect of MOAG-4

might also be due to the short timeframes of *C. elegans* protein aggregation models. In this scenario, slower aggregation pathways might be less dependent on MOAG-4.

Chapter 3 of this thesis contains the description of a method developed for the native screening of fluorescently labelled aggregation-prone proteins. The method is based on the separation of fluorescently labelled proteins within biological samples through an agarose gel and their analysis using a fluorimetric scanner. The chapter also describes how to combine this method with others to characterise protein species of interest. The method was developed to analyse the effect of modifiers of aggregation on the aggregation process, but would also be suitable for monitoring other protein modifications, cleavage products or oligomerisation. The main strengths of the method are the speed and low workload of the initial step which allows for a quick screening of experimental conditions and reliably monitors any changes in the distribution of protein species. Since the method is non-denaturing, it will detect proteins in complexes which can complicate analysis but also be used to follow complex states under different conditions. The initial electrophoresis step in a 1 % agarose gel consistently retains microscopically visible protein aggregates in the loading wells, because no significant alteration in the ratio of retained fluorescent signal with or without pre-separation of aggregates by centrifugation was detected and it also correlates to aggregates quantified *in vivo*. This removes the need of a separate dot-blot for aggregate retention and the method does not suffer from the same problems with signal linearity detection as for instance horse radish peroxidase, although some loss can be unavoidable due to fluorescent quenching.

Many experiments aimed to increase our understanding of the process of amyloidosis require purified protein, both those prone to form amyloids as well as the network of proteins interacting with them. Chapter 4 of this thesis describes the use of yeast homologous recombination to facilitate flexible and quick creation of expression vectors for protein purification. The SUMO-tag used serves both as a way to produce proteins free from unwanted restriction site residues and as a solubility tag with can strongly increase yields of aggregation prone proteins. The author hopes that the work presented herein will increase our understanding of protein misfolding especially though the novel function of Moag-4 and that the methods described will lead to faster results and broader approaches regarding protein misfolding and other fields of research.